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# Orthotopic Induction of CH157MN Convexity and Skull Base Meningioma into nude mice using stereotactic surgery and MRI Characterization.

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## Abstract

Meningioma *in vivo* research is slowed by the difficulty of establishing an easy and reproducible orthotopic model able to mimic the characteristics of a human meningioma. Moreover, leptomeningeal dissemination and high mortality is often associated with this kind of orthotopical models, making them useless for clinical translation studies.<sup>1-2</sup> An optimized method to induce meningioma on nude mice in two different sites is described in this paper, proving its high reproducibility and low mortality. Skull Base Meningioma was induced into the auditory meatus, while the Convexity was induced on the brain surface of 23 and 24 nude mice, respectively. Both models lead to the development of a mass easily observable by imaging methods. Dynamic Contrast Enhanced (DCE) MRI was used as a tool to monitor and characterize the pathology onset and progression. At the end of the study, histology was performed to confirm the neoplastic origin of the diseased mass.

## Introduction

This study focuses on the induction of two orthotopic meningioma models: Skull Base of the auditory meatus, and Convexity Meningioma. The models were selected as an example of meningioma tumor in mice, to be used in preclinical studies for translational research, being meningioma one of the brain tumors with the highest incidence. Despite a relatively low mortality characterizes human meningioma, the development of early diagnostic tools and/or of effective therapies is extremely important to improve the outcome of the medical treatment of such pathology,

Both models were induced by using CH157MN cell line, isolated for the first time in 1977 from a 41 years old woman<sup>3</sup>. This line was selected among others because of its ability to reproduce histological, immunohistochemical and structural features of human meningioma. CH157MN model was already introduced by Ragel et al in 2007 and 2008<sup>4-5</sup>; nevertheless the papers were focused on the model characterization via luciferase rather than on the description of the induction or on the characterization by MRI (tumors were imaged post mortem by MRI, only to calculate tumor volume, rather than to have a precise localization and description of the model in real time). Similarly, Giogigeni and Karsy<sup>6-7</sup> adopted the model, but they both use bioluminescence imaging to monitor tumor growth and response to treatment. In such manner it was possible to approximately know if the treatment was reducing the tumor mass, but not the precise localization and characteristics, since bioluminescence imaging lacks in resolution, not allowing to accurately measure the tumor burden, its depth, and localization.

47 athymic female nude mice (5-6 weeks old) were inoculated with CH157MN cells and imaged weekly on a 1 T scanner, before and after administration of a commercial Gadolinium Based Contrast Agent (GBCA). The introduction of a non-invasive method as MRI, allowed a proper evaluation of tumor growth, vascularization and GBCA perfusion/permeability.

## PROTOCOL

All the procedures involving animals were conducted according to the national and international laws on experimental animals (L.D. 26/2014; Directive 2010/63/EU). This experimental protocol was approved by the Italian Ministry of Health with Authorization 724/2017 PR.

CH157MN cells were cultured in DMEM F12 with 7% FBS. For the induction, they were resuspended in 3-8  $\mu$ L of serum-free DMEM F12.

### Tumor Induction

Mouse was subcutaneously administered with carprofen 5 mg/kg one hour before the surgery. Anesthesia was induced with sevoflurane gas and then systemically by Rompun<sup>®</sup> 5 mg/kg and Zoletil<sup>®</sup> 40 mg/kg. Mouse was then mounted on the stereotaxic apparatus and its temperature was continuously monitored and maintained in the range 36.5-38.5 °C. After cleaning the skin with a disinfectant, a local anesthesia was administered. Bregma was then exposed and the site of induction was identified by using the following coordinates: 3 mm anterior, 2 mm lateral to bregma and 2 mm under the frontal bone for the Convexity Meningioma and at the skull base (13-14 mm) for Skull Base Meningioma. A small hole was drilled manually, and a volume of 3-8  $\mu$ L containing  $5 \times 10^4$  -  $5 \times 10^5$  (skull base) to  $10^5$ - $10^6$  (convexity) CH157MN cells was released manually in approx. 1  $\mu$ L/3 min by using a Hamilton syringe with 25G needle. The hole was closed after removing the needle, and the mouse removed from stereotaxic apparatus. For more details see the supplementary material.

### Imaging Protocol

Mice were imaged once or twice a week using a 1 T Icon (Bruker) scanner. After preliminary anatomical scans, MSME sequences were acquired before and after the intravenous administration of the GBCA. The following parameters were set: Matrix size = 128 x 128, FieldOfView = 1.6 x 1.6 cm, slice thickness 1.2 mm, TE(echo time)= 9.2 ms, TR(repetition time)= 350 ms, acquisition time = 90 s, NA = 2.

## Results

CH157MN meningioma was induced on 47 nude mice, according to the experimental protocols summarized in Table 1. The induction was successful in 35/47 animals, specifically in:

- 15/24 for Skull Base Meningioma (4 mice did not survive the systemic anesthesia and 1 showed clinical signs immediately after the surgery; 4 mice did not develop any tumor);
- 20/23 for Convexity Meningioma (1 mouse did not survive the systemic anesthesia; 2 mice did not develop any tumor).

As detailed in Table 1, once the inoculation site has been set, different experimental conditions were tested (*i.e.* the cell number and the method adopted to suture the surgical wound) to optimize the protocol.

The Convexity Meningioma generally showed a better outcome than the Skull Base model, either in terms of success of the surgery procedure and in terms of animal welfare. Specifically, the intra-operative mortality was lower for 3 over 4 groups inoculated superficially, indicating as expected a minor surgery risk, since the needle stops at the surface rather than passing through the brain to reach the auditory meatus. Even after surgery, the occurrence of clinical signs was more severe for the Skull Base model, with the appearance of unsteady gait, domed head and loss of equilibrium, this latter probably due to the specific location of the tumor in the auditory meatus. As a further advantage of the Convexity model, the tumor take rate was superior. Tumor volume, growth rate and maximum enhancement after GBCA administration were comparable between the two sites. All the different suture procedures adopted for both the Skull Base and Convexity Meningioma were effective to avoid the appearance of any extra-cranial mass.

Needle with tip was used during cells inoculation only for three animals, while for all the other experimental groups the needle without tip was used. This choice was motivated by the fact that, only one animal over three inoculated using a needle with tip developed an observable tumor mass; most likely the tip avoided a proper release of tumor cells.

One week after tumor induction, animals without significant or prolonged clinical signs were imaged weekly (or closer) by MRI and administered with a GBCA in the presence of a clearly visible tumor mass. A tumor mass was generally visible starting from 1 week/10 days from induction in both inoculation sites, as observable for representative animals in all geometrical section of T<sub>2</sub>-weighted images (Figure 1, A and B). T<sub>1</sub>-weighted images (pre and post contrast) together with signal enhancement vs time quantification are shown in panel A and B of Figure 2 for Skull Base and Convexity meningioma, respectively. In pre contrast images, brain tissue and tumor mass showed the same signal, but after GBCA administration the diseased tissue reached a higher enhancement and became clearly discernible from the contralateral region, *i.e.* auditory meatus for Skull Base Meningioma and healthy brain for Convexity Meningioma. The enhancement of the tumor mass was always noticeable, ranging from 50 to 120 % with few exceptions at short times after induction or when the tumor mass was not yet well developed.

Finally, meningioma identity and tissue vascularization were assessed by histological analysis. Both CH157MN orthotopic tumors exhibited histological analogies of human grade III meningioma tumor, such as nuclear polymorphism, large cells with eccentric nuclei and abundant cytoplasm and mitosis (Figure 3). The tumors displayed infiltrative growth, widespread vascularization and, often, the presence of hemorrhages.

## Discussion

The induction of two different models of orthotopic meningioma into nude mice was described.

Skull Base Meningioma was correctly induced into the auditory meatus of female nude mice. The most critical steps for the appropriate induction of the meningioma into this site were:

- 1) Ventral coordinate optimization to the skull base. The best coordinate was identified based on the fact that the needle encountered resistance when moved in the ventral position and resulted to be 14 mm. This value stays true if the mouse used has the same strain, sex and age we described.
- 2) Rate of cellular release into the auditory meatus. The volume needed to suspend the cells is really high, so it is important that the release happens slowly and carefully. Else, an accumulation of edema into the auditory meatus could cause massive clinical signs such as loss of equilibrium at the end of the procedure.

Convexity Meningioma was easier to induce and overall showed less clinical signs and troubleshooting. However, these steps should be performed with particular care for the appropriate induction of the model:

1. Dura mater preservation. Dura mater should not be damaged when drilling the skull, so the hole was drilled manually, with the use of magnifying glasses to help the operator. When the drill encounters less resistance, this is when the drilling has to stop.
2. Tumor growth outside the skull. It is important to close the hole in the proper way to avoid extracranial tumor growth. Dental cement proved to be the more efficient choice to close the hole correctly.

Non-invasive MRI was introduced to characterize in real time the evolution of the cerebral pathologies, even from early stages. The onset of the pathologies was monitored at different time points, allowing the operator to control the appearance and growth of the disease, as well as the permeability properties, by injection of a GBCA.

The feasibility and high reproducibility of the pathological models proved them to be effective for GBCA validation MRI studies. The precise location of the meningioma tumors as seen on MRI proved to mimic the complex situations in human beings<sup>8</sup>. The noticeable enhancement obtained after GBCA administration in the investigated orthotopic meningiomas demonstrated that these models could be used as a “bridge” to fill the gap between clinical and preclinical efficacy studies.

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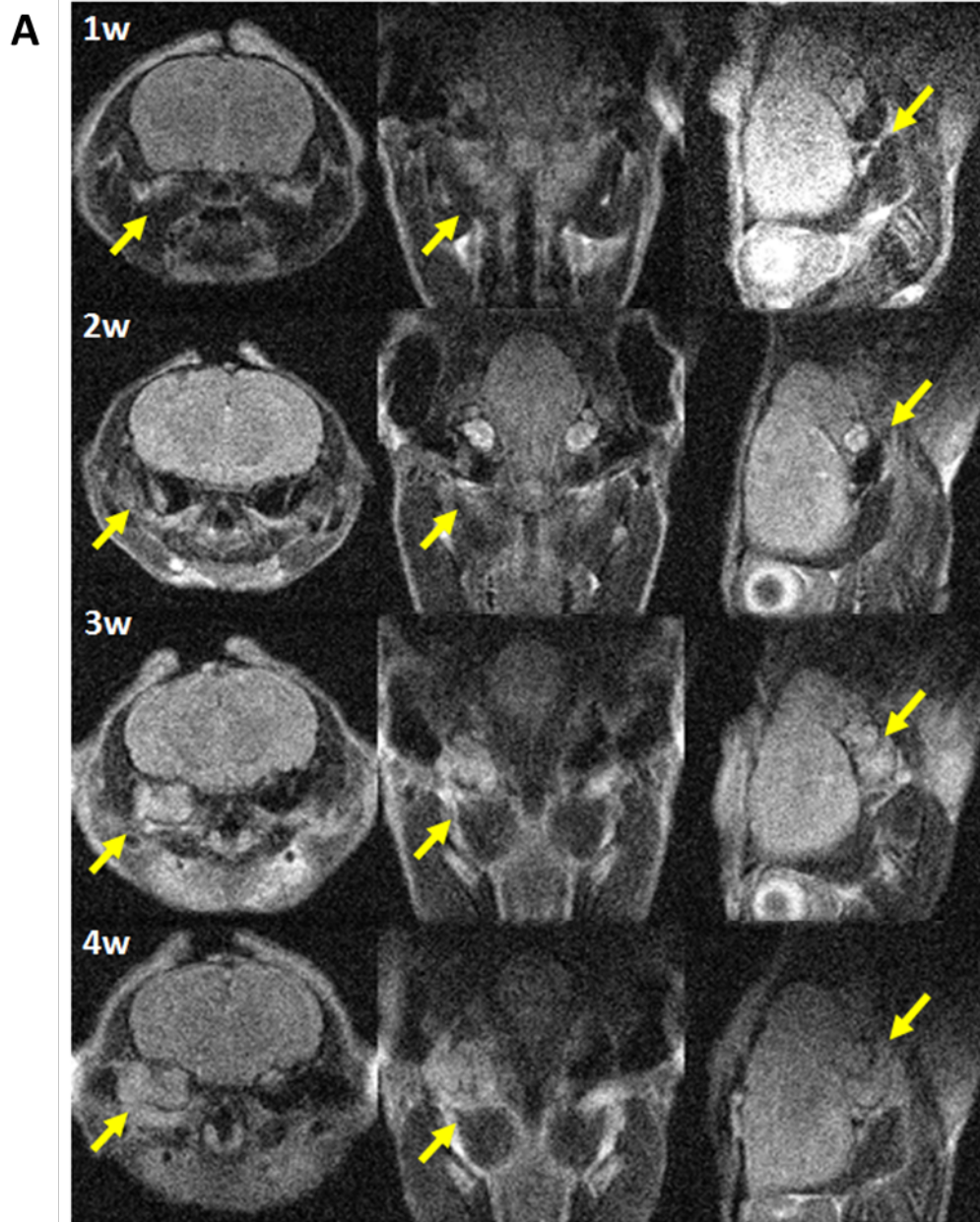
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**Table 1: Experimental Scheme and results.**

| Group | Total Animals | Cell Number/Suture Procedure  | Site       | Stereotaxic Coordinates   | Intraoperative Mortality | Tumor Take Rate | Average Tumor Volume (mm <sup>3</sup> ) | Average Maximum Enhancement (%) | Clinical Signs   |
|-------|---------------|---|------------|---|--------------------------|-----------------|---|---------------------------------|--|
| 1     | 6             | 5·10 <sup>4</sup> suspended in 3 µL, surgical glue to seal/suture both the skull hole and the wound | Skull base | Ant. 3 mm, Lat. 2 mm, Vent. 12-13 mm and needle with tip; Ant. 3 mm, Lat. 2 mm, Vent. at skull and needle without tip | 1/6                      | 4/5             | 55                                      | 80                              | Neurological damage, unsteady gait, tumbling, apathy   |
| 2     | 6             | 5·10 <sup>5</sup> suspended in 3 µL, surgical glue to seal/suture the skull hole and the wound      | Skull base | Ant. 3 mm, Lat. 2 mm, Vent. at skull and needle without tip   | 1/6                      | 3/5             | 85                                      | 70                              | No observations  |
| 3     | 6             | 5·10 <sup>4</sup> suspended in 3 µL, silk thread (5.0) to suture the wound                          | Skull base | Ant. 3 mm, Lat. 2 mm, Vent. at skull and needle without tip   | 1/6                      | 2/5             | 45                                      | 70                              | Loss body weight <8%, dyspnea, isolation from the group, dehydration, closed eye lids, unsteady gait |

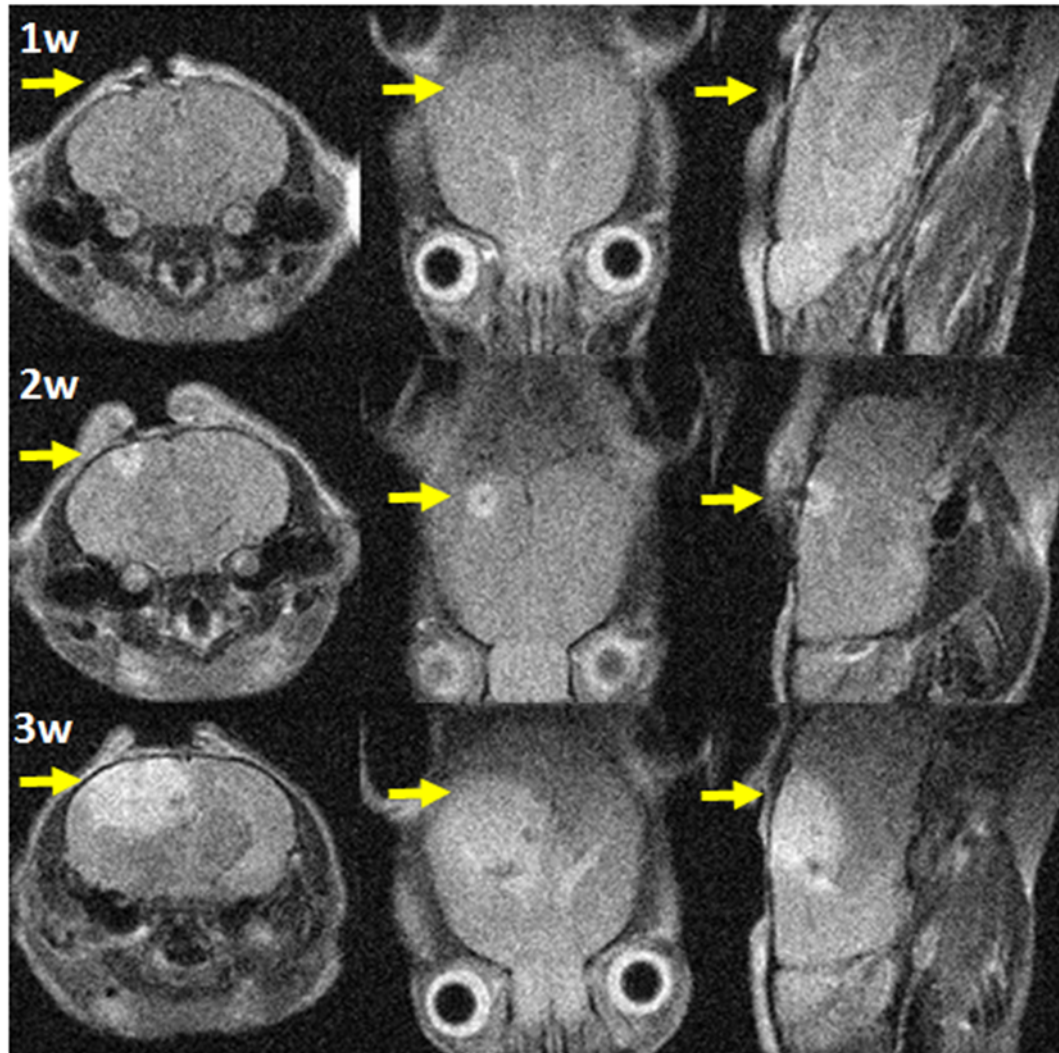
|   |   |  |            |   |     |     |    |    |   |
|---|---|--|------------|---|-----|-----|----|----|---|
| 4 | 6 | 5·10 <sup>5</sup> suspended in 5 µL, silk thread (5.0) to suture the wound                                     | Skull base | Ant. 3 mm, Lat. 2 mm, Vent. at skull and needle without tip | 2/6 | 4/4 | 50 | 55 | Tachipnea, isolation from the group, immobility, closed lid eye, loss of coordination and equilibrium |
| 5 | 6 | 10 <sup>5</sup> suspended in 3 µL, surgical glue to seal/suture the skull hole and the wound                   | Convexity  | Ant. 3 mm, Lat. 2 mm, Vent. 2 mm and needle without tip     | 1/6 | 5/5 | 62 | 70 | Domed head  |
| 6 | 5 | 10 <sup>6</sup> suspended in 8 µL, surgical glue to seal/suture the skull hole and the wound                   | Convexity  | Ant. 3 mm, Lat. 2 mm, Vent. 2 mm and needle without tip     | 0/5 | 4/5 | 50 | 70 | No observations   |
| 7 | 6 | 10 <sup>5</sup> suspended in 3 µL, bone wax to seal the skull hole and silk thread (5.0) to suture the wound   | Convexity  | Ant. 3 mm, Lat. 2 mm, Vent. 2 mm and needle without tip     | 0/6 | 5/6 | 40 | 80 | Domed head and retracted eyes   |
| 8 | 6 | 10 <sup>6</sup> suspended in a 6 µL, bone wax to seal the skull hole and silk thread (5.0) to suture the wound | Convexity  | Ant. 3 mm, Lat. 2 mm, Vent. 2 mm and needle without tip     | 0/6 | 5/6 | 50 | 60 | Domed head and retracted eyes   |

**Figure 1.** Representative anatomical T<sub>2</sub>-weighted images at different weeks post tumor induction in all geometrical sections (from left to right axial, coronal and sagittal). A: Skull Base meningioma. B: Convexity Meningioma.

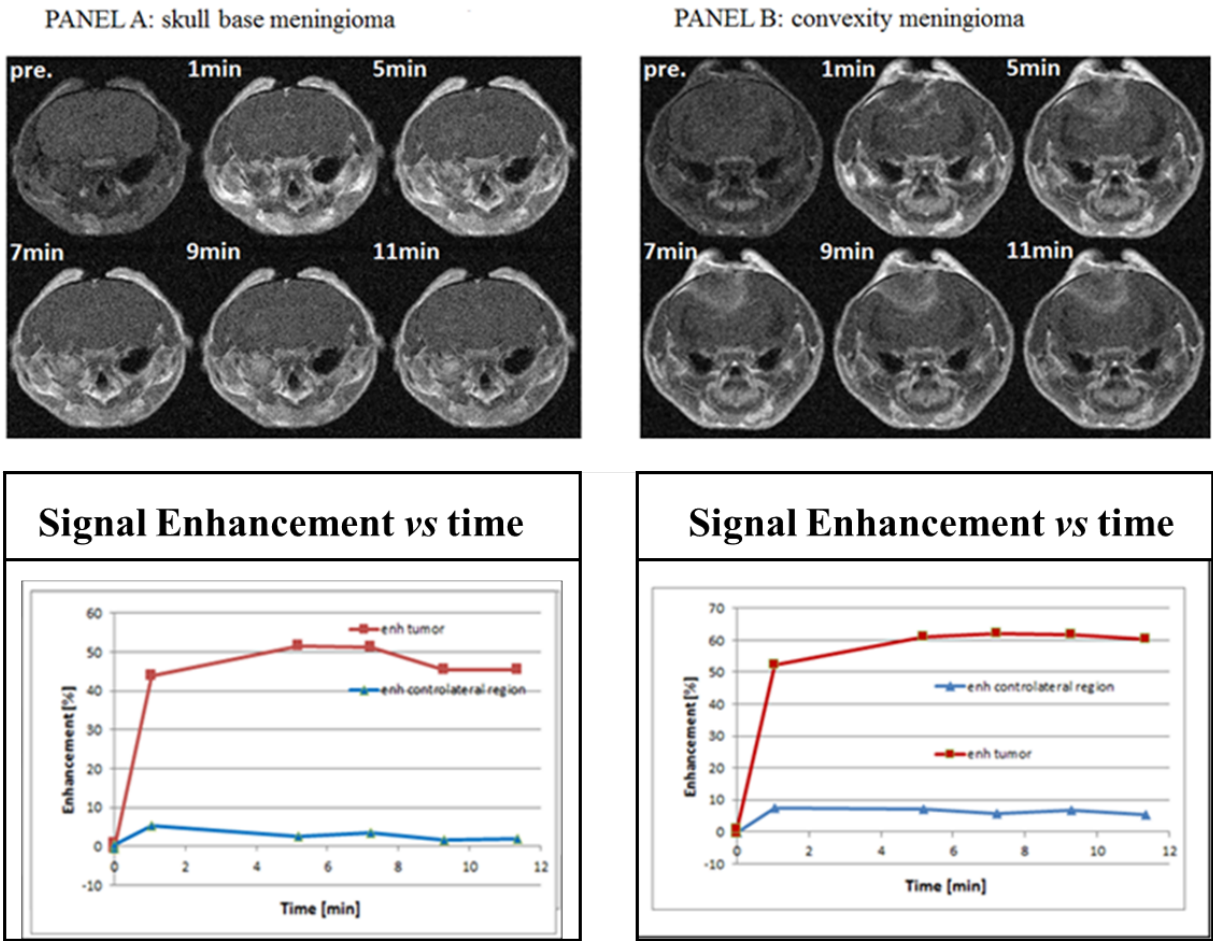




B



**Figure 2.** Representative contrast enhanced T<sub>1</sub>-weighted images and MSME signal enhancement as a function of time for healthy contralateral tissue (blue) and tumor mass (red).



**Figure 3.** Representative examples of histological images of Skull Base Meningioma (A) and Convexity (B).

